RELATIONSHIP BETWEEN *DIGITARIA MILANJIANA* (POACEAE: PANICEAE) AND THE ANNUAL SPECIES OF *DIGITARIA* SECTION *DIGITARIA* IN NORTH AMERICA[1]

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ABSTRACT

Morphological, anatomical, and cytological data were used to show the taxonomic relationship between the perennial *Digitaria milanjiana* and the annual species of *Digitaria* section *Digitaria* (*D. bicornis, D. ciliaris, D. horizontalis, D. nuda, D. saguinalis* and *D. setigera*). A phenetic analysis showed populations of *D. milanjiana* form a distinct cluster separate from the most closely related annual (*D. bicornis*), and a statistical analysis showed these species differed significantly on 16 of the 20 characters measured. A nested analysis of variance gave corresponding results. Instead of characters pertaining to longevity, it is suggested that the total absence of leaf pubescence, the presence of spicules on the lower lemma nerves, a narrow lateral branch, and a short and narrow bract blade be used to segregate *D. milanjiana* from the annual taxa. An analysis of North American populations of *D. milanjiana* showed no justification for the recognition of subspecific categories of this species. In addition, general shape and average size of the epidermal long cells and the association of sclerenchyma tissue with second-order vascular bundles differed between the annual taxa and *D. milanjiana*. Cytologically, *D. milanjiana* was found to be both male and female sterile, whereas the annuals were highly male fertile and produced a normal embryo sac. It was concluded that a measurable and definable difference exists between *D. milanjiana* and the annual members of the section *Digitaria.*

Stapf (1919) transferred *Panicum milanjiana* Rendle to *Digitaria* and placed this species in the subsection *Sanguinales*. At that time Stapf recognized two sections and nine subsections of *Digitaria* and considered spikelet pubescence patterns to be taxonomically important at subgeneric levels. Included within the *Sanguinales* were both robust perennials and weak decumbent annuals. In his monographic work of *Digitaria*, Henrard (1950)

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segregated Stapf's *Sanguinales* into four sections which included the *Erianthae, Horizontales, Parviglumae*, and the original *Sanguinales*. Henrard restricted the *Erianthae* to the robust perennials native to Africa, whereas most of *Sanguinales* were annuals which lacked stolons. Henrard distinguished the *Parviglumae* and *Horizontales* from the *Sanguinales* on the relative size of the glumes and inflorescence characteristics. These sections were realigned by Veldkamp (1973), who recognized the section *Digitaria* within the subgenus *Digitaria* to encompass the *Horizontales, Parviglumae, Sanguinales*, and *Biformes*. North American members of these sections intergrade (Webster 1981), and the concept of combining them into one section is certainly justified. Veldkamp (1973) maintained the *Erianthae* as a separate section. Agrasar (1974) in her treatment of *Digitaria* in Argentina combined the *Sanguinales* and *Erianthae* (*D. decumbens* Stent) into the section *Digitaria*. Agrasar's treatment represented a reversal to Stapf's (1919) original subgeneric concept.

Members of African *Erianthae* have been widely introduced into temperate and tropical regions of the world as grasses for improved pastures. The most important of these species included *D. pentzii* Stent, *D. decumbens*, *D. eriantha* Steudel, and to a lesser degree *D. milanjiana*. Recent studies of *Digitaria* have shown that *D. milanjiana* occurs in North America even though it has been unreported from this hemisphere. This species was found to dominate the sandy coastal plain of eastern Mexico from just south of Ciudad Victoria to Villa Hermosa, and specimens were observed from the Caribbean and Central America. It also has escaped cultivation and occurs sporadically in southern Texas and Florida. Hodges, et al. (1975) stated that *D. decumbens* was first introduced into the United States in 1935 from Pretoria, South Africa, and was planted on an estimated one million acres on the Gulf Coast of Mexico. Based on its present distribution, it seems likely that this introduction was actually *D. milanjiana* rather than *D. decumbens*.

Digitaria milanjiana is morphologically closely related to the annual species of the section *Digitaria* [*D. bicornis* (Lamk.) R&S, *D. ciliaris* (Retz.) Koeler, *D. horizontalis* Willd., *D. nuda* Schum., *D. sanguinales* (L.) Scopoli, and *D. setigera* Roth.]. Webster and Hatch (1981) defined this complex as consisting of annuals with paired lanceolate spikelets on a winged primary branch. *Digitaria milanjiana*, as do other members of the *Erianthae*, has the identical spikelet shape, arrangement, and inflorescence characteristics of the annual species. The identification of this species is further complicated by the fact that the perennial nature of *D. milanjiana* is seldom represented on herbarium sheets. Specimens were found to be frequently misidentified as *D. ciliaris*, *D. bicornis*, or even *D. sanguinales*. The objectives of the present study were to: 1) report the occurrence of *D. milanjiana* in North America and to give a detailed morphological description of this species, 2) phenetically and statistically compare *D. milanjiana* to the most closely related annual species based on characters considered to be of taxonomic importance within the annual complex, and 3) anatomically and cytologically compare *D. milanjiana* to members of the annual complex.

METHODS

Collections of *Digitaria* were made in the eastern United States and throughout Mexico. A total of 245 populations were sampled, including 17 populations of *D. milanjiana* and 228 populations of the annual complex. At each collection site, ten independent specimens were collected and pressed. To insure isolation between adjacent populations, at least 20 km were travelled between collection sites. All specimens from the 245 populations or 2,450 specimens were used in the numerical analysis. On each specimen measurements of 20 characters (Table 1) were recorded. These included seven spikelet characters, seven inflorescence characters, and six characters from the vegetative part of the specimens. It should be noted that characters pertaining to the perennial nature of *D. milanjiana* were not recorded as it was an objective to distinguish this species based only on those characters considered taxonomically important within the annual complex.

A variety of numerical techniques were employed to delimit and define the phenetic and statistical relationships among the populations. Data were analyzed using the Statistical Analysis System (SAS) (Barr, et al. 1976) and the Numerical Taxonomic System of Multivariate Statistical Programs (NT-SYS) package (Rohlf, et al. 1971). Correlation and distance phenograms, principal components analysis (PCA), and multivariate analysis of variance (MANOVA) (Schmidly and Hendricks 1976) were used to show the phenetic relationship among the populations. Basic statistical procedures were used to analyze the variation between and within the population clusters. These clusters were compared on the basis of character means, ranges, standard deviations, coefficients of variation, and F-values. A nested analysis of variance procedure was employed to give the relative percent variation due to differences between taxa, among populations, and among specimens within populations. Correlation coefficients were used to show the relationship between characters for a species. Finally, discriminant analysis was used to test the distinctiveness of the taxa.

Material for anatomical and cytological examination was collected for each of the populations. Live material was killed and fixed in 3:1 (V/V) ethanol and glacial acetic acid for 12 hours, transferred to 70 percent ethanol and refrigerated until examination. Adaxial epidermal peels of leaf blades were made by scraping away the abaxial surface and extraneous matter with a razor blade, cleared in full strength bleach for 5 minutes, and viewed with a light microscope with phase-contrast. Ovaries were carefully dissected from the upper floret, cleared with methyl salicylate (Young, et al. 1979), and observed under phase-contrast. Pollen fertility counts were made by staining the pollen with cottonblue in lactophenol (Radford, et al. 1974).

Table 1. Variables measured for population analysis.

	VARIABLE	ABBREVIATION	PRECISION
1	Spikelet length	(SL)	0.1 mm
2	Spikelet width	(SW)	0.01 mm
3	Length of first glume	(LFG)	0.01 mm
4	Length of second glume	(LSG)	0.1 mm
5	Distance to lateral nerve on sessile spikelet	(DLS)	0.01 mm
6	Distance to lateral nerve on pedicelled spikelet	(DLP)	0.01 mm
7	Spicules on lateral nerves	(SN)	0.01 mm
8	Pedicel length	(PL)	0.1 mm
9	Lateral branch length	(RL)	1.0 mm
10	Lateral branch width	(RW)	0.1 mm
11	Length of main axis	(LMA)	1.0 mm
12	Number of lateral branches	(LB)	—
13	Number of spikelets	(NS)	—
14	Length of setaceous hairs on lower pulvinus	(LH)	0.1 mm
15	Length of bract blade	(LLB)	1.0 mm
16	Width of bract blade	(WBB)	1.0 mm
17	Length of bract sheath	(LBS)	1.0 mm
18	Setaceous hairs in the ligule region	(SLH)	—
19	Ligule length	(LL)	0.1 mm
20	Distance of pubescence up sheaths	(DP)	1.0 mm

Pollen grains completely filled with blue stain were classified as viable, whereas pollen with no stain or partially stained were classified as inviable.

RESULTS AND DISCUSSION

A phenetic analysis of all populations collected showed *D. milanjiana* to be most closely allied to *D. bicornis* (taxonomic relationships among members of the annual complex are to be the topic of a future publication and will not be discussed here). Populations of *D. milanjiana* were clustered with those of *D. bicornis* as a result of a number of morphological similarities. These similarities included the distance to the lateral nerves on the lower lemma, small or nonexistent main axis, few setaceous hairs in the ligular region, spikelet length, and length of the second glume. The phenetic relationship among the 17 populations of *D. milanjiana* and 43 populations of *D. bicornis* is shown by a correlation phenogram ($r = 0.91$) (Figure 1). The OTU's correspond to population collection numbers of the senior author (collections are on deposit at L, MO, NY, TAES, and US). This analysis showed two distinct population clusters. The smaller cluster contained the perennial species and showed relatively little variation among the populations. Populations of the annual species comprised the larger cluster. Poputions of *D. milanjiana* were collected in eastern Mexico from Ciudad Victoria to Villa Hermosa where it was found to be abundant. *Digitaria bicornis*

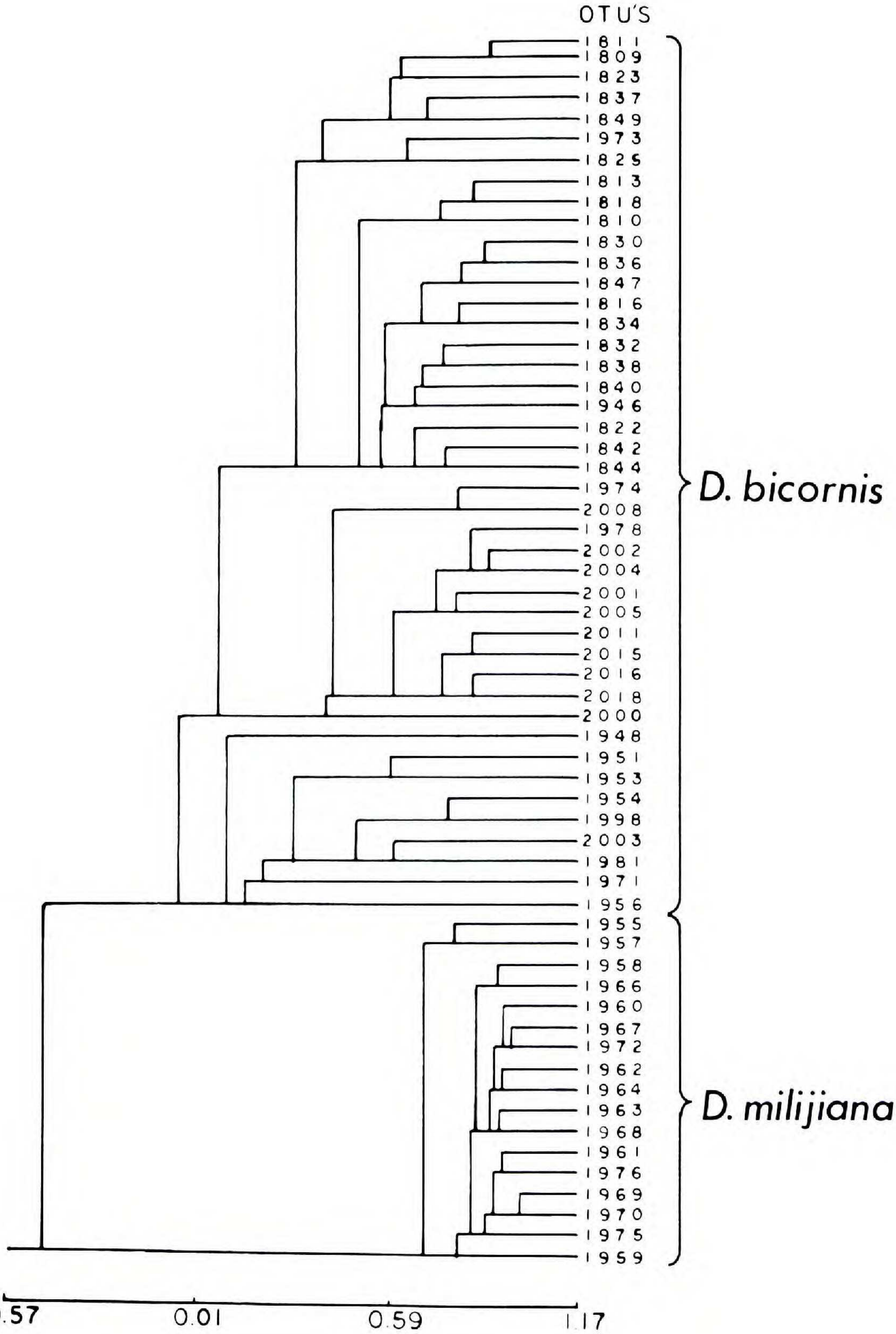

Fig. 1. Correlation phenogram (r = 0.91) of 43 populations of *Digitaria bicornis* and 17 populations of *Digitaria milanjiana*.

populations were collected in the southeastern United States (Webster 1980) and essentially throughout Mexico.

PCA on these populations also showed a clear separation of the taxa. Approximately 60 percent of the variation was accounted for by the first two components. Populations of *D. milanjiana* were separated from those of *D. bicornis* based on characters contributing to the first component. The most important characters (importance of the characters was based on the relative size of the intracomponent correlations) were spikelet width, lateral branch length, bract blade length, and second glume and ligule length.

A basic statistical comparison (Table 2) was made between the 17 populations of *D. milanjiana* and the 20 most closely related annual populations of *D. bicornis*. *Digitaria milanjiana* differed significantly ($p < 0.0001$) from the annual on 16 of the 20 measured characters. Length of the second glume, spikelet width, and length of the first glume were spikelet characters with the highest F-values. Range overlaps existed for each of these characters. Spikelets of *D. milanjiana* are lanceolate with villous hairs between the lateral nerves of the seven nerved lemma of the lower floret (Figure 2). Minute scabrosities are present on all nerves of the lower lemma, which is a characteristic that can be consistently used to separate this species from other members of the *Erianthae*. The first glume is triangular in shape and glabrous, whereas the second glume is lanceolate, three nerved, pubescent (Figure 3). Four characters of the inflorescence were significant, the most

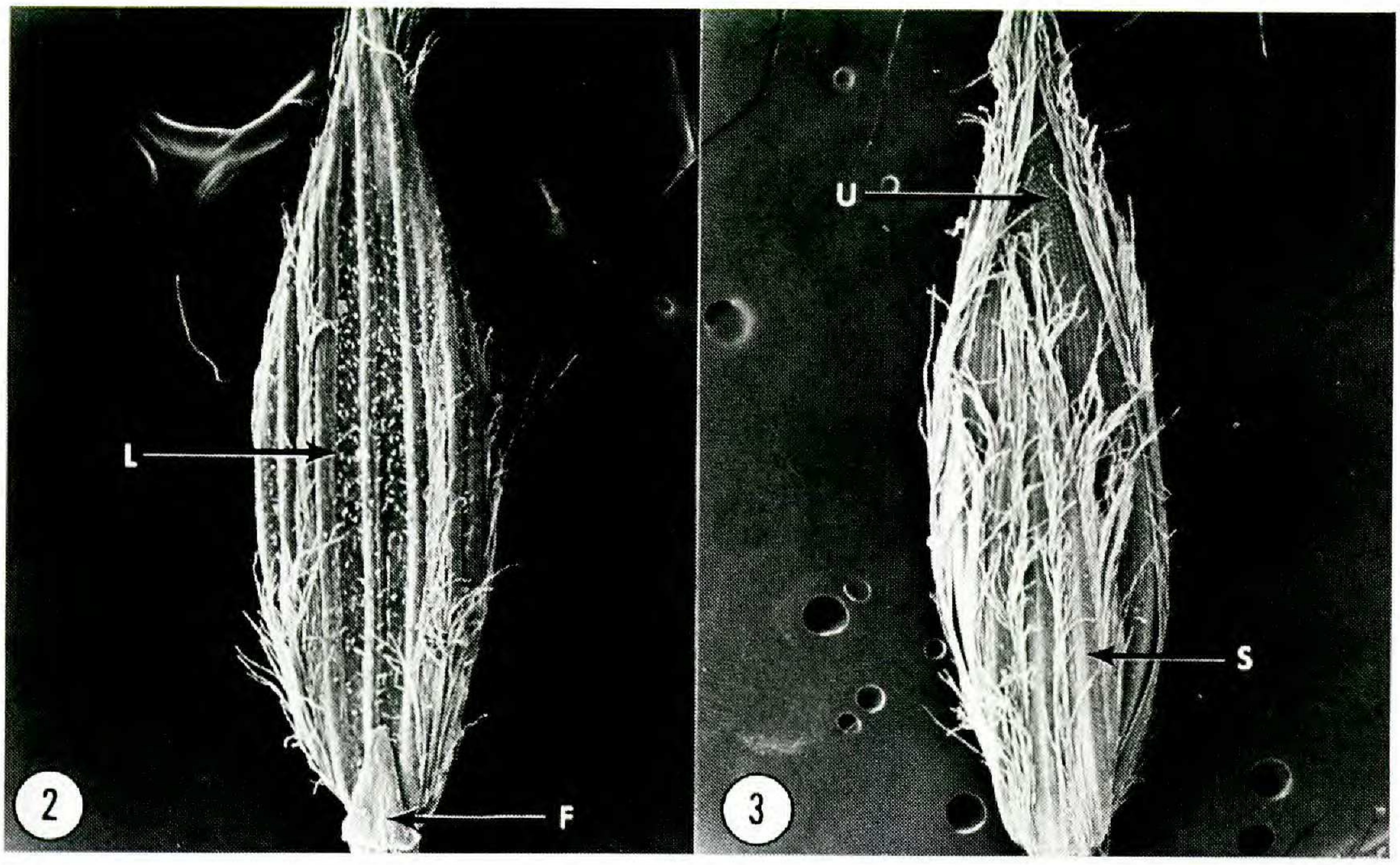

Fig. 2-3. Scanning electron micrographs of the spikelet of *Digitaria milanjiana* (Webster 1955, ca 20 X). Fig. 2. Abaxial orientation of the spikelet showing the first glume (F) and lemma of the lower floret (L). Fig. 3. Adaxial orientation of the spikelet showing the second glume (S) and lemma of the upper floret (U).

Table 2. Means, ranges, standard deviations, coefficients of variation, and F-values for 20 characters of *D. milanjiana* and *D. bicornis*. Distance given in millimeters.

	Digitaria milanjiana N = 170				*Digitaria bicornis* N = 199				
	MEAN	RANGE	S	C.V.	MEAN	RANGE	S	C.V.	F-VALUE
SL	2.98	2.5-3.3	0.14	4.64	3.21	2.6-3.6	0.19	5.77	173.2*
SW	0.78	0.67-0.90	0.05	6.28	0.89	0.73-0.99	0.06	6.52	440.3*
LFG	0.36	0.23-0.47	0.05	13.23	0.27	0.00-0.47	0.06	21.29	304.8*
LSG	1.76	1.5-2.2	0.12	6.65	2.32	1.8-2.8	0.21	8.93	973.8*
DLS	0.20	0.17-0.27	0.24	12.17	0.19	0.13-0.27	0.02	11.53	5.2
DLP	0.20	0.13-0.30	0.25	12.18	0.22	0.17-0.27	0.03	11.68	62.5*
SN	0.83	0.0-2.9	0.93	112.88	0.17	0.0-1.3	0.33	215.18	101.4*
PL	2.67	1.4-3.1	0.32	14.76	2.19	1.4-4.2	0.38	17.42	0.5
RL	126.18	70-169	18.43	14.60	139.98	71-206	25.18	17.99	35.8*
RW	0.56	0.4-0.8	0.08	13.55	0.95	0.8-1.3	0.14	14.73	1098.4*
LMA	0.42	0-12	1.89	453.16	2.50	0-40	7.79	311.19	13.2
LB	0.01	0-1	0.10	919.22	0.10	0-3	0.41	412.26	7.5
NS	12.18	9-19	1.83	15.04	7.86	5-13	1.47	18.72	679.0*
LH	0.01	0.0-0.3	0.04	605.11	0.06	0.0-0.7	0.11	178.49	45.9*
LBB	28.18	6-126	17.07	60.59	69.46	21-249	33.26	47.88	183.4*
WBB	1.80	0-6	0.97	53.64	4.49	2-9	1.15	25.69	566.9*
LBS	180.34	60-242	26.03	14.44	138.18	82-228	27.01	19.55	253.2*
SHL	—	—	—	—	8.82	0-66	5.12	58.05	487.4*
LL	1.10	0.4-2.2	0.28	25.07	2.05	1.0-3.5	0.43	21.27	604.1*
DP	—	—	—	—	32.46	0-78	16.20	49.90	606.8*

* $P < 0.0001$

important of these included a narrower lateral branch and more spikelets per unit length of lateral branch. Pedicels of *D. milanjiana* are triangular in cross section with scabrosities on the angles. The wing of the lateral branch is only slightly smaller than the body and distinctly scabrous on the margins. This species has digitate lateral branches and only rarely will possess a main axis. The lowermost pulvinus is covered with short hispid hairs. *Digitaria milanjiana*, as it exists in North America, can easily be distinguished from members of the annual complex based on the glabrous nature of the leaves. Papillose-based setaceous hairs are absent from the throat of the ligular region which is a characteristic of the annual complex. The first leaf subtending the inflorescence (bract blade) of the perennial was found to be significantly shorter and narrower than those of the annuals. Finally, the ligule of *D. milanjiana* was significantly shorter than other species examined.

A nested analysis of variance procedure was employed to further illustrate the statistical relationship existing between these species. This procedure gives the relative percent variation due to specimens within populations, among populations, and btween taxa. Ideally, if two distinct species are being compared then a higher percent of the variation will be accounted for at the species level. Eleven of the 20 characters (Table 3) had more

Table 3. Relative percent variation due to differences among specimens, within populations, among populations, and between taxa of *D. milanjiana* and *D. bicornis*.

	SPECIMENS	POPULATIONS	TAXA
SL	29.46	23.83	46.71
SW	26.53	5.34	68.13
LFG	30.56	8.27	61.17
LSG	8.22	8.13	83.65
DLS	86.06	10.37	3.56
DLP	71.15	7.11	21.74
SN	60.19	8.34	31.47
PL	74.65	25.35	0.00
RL	58.12	27.47	14.32
RW	12.04	2.58	85.37
LMA	46.20	50.80	3.00
LB	88.16	8.92	2.92
NS	13.59	9.49	76.92
LH	79.97	6.78	17.25
LBB	26.25	21.06	52.69
WBB	15.93	8.70	75.37
LBS	32.75	12.21	55.05
SHL	21.83	5.12	73.05
LL	17.13	6.61	76.26
DP	16.17	5.29	78.54

than 50 percent of the variation due to differences between species. These were the same characters possessing the highest F-values (Table 2). Except for length of the main axis (LMA), a relatively low percent of the variation was accounted for at the population level. A discriminate analysis procedure was used to test the distinctness of *D. milanjiana* and *D. bicornis*, and there was no misclassifications of the 369 specimens tested.

Correlation coefficients were used to show the relationship between characters of a species. Significant ($P < 0.0001$) correlation coefficients existed between 18 character combinations of *D. milanjiana*. Highest positive values were between PL-SL, RL-LBS, and WBB-LBB. Significant negative correlations existed between NS-PL and between NS-RL. As lateral branches increase in length, the number of spikelets per unit area decrease, thereby showing a consistency as to the number of spikelets on a lateral branch.

Henrard (1950) recognized one subspecies and one variety of *D. milanjiana*. The question then arises as to the possibility of subspecific categories of this species in North America. The first three vectors of a MANOVA procedure on populations of *D. milanjiana* accounted for 58.9 percent of the variation, which is relatively low for this type of analysis and is indicative of a homogeneous group of populations. A separation of any group of populations did not exist. An analysis of variance on these 17 populations showed that there were significant differences ($P < 0.0001$) among six of the characters measured. These included SL, PL, RL, RW, NS, and LL. However, the highest F-value was only 6.39. Duncan's multiple range test showed that these differences could not be correlated to any specific group of populations. For example, whereas one group of populations might differ significantly from other populations on SL, the same group would not be significantly different for any of the other characters. It was concluded that these differences were the expected variation among populations over a wide geographic area and, thereby, taxonomically insignificant. Based on North American specimens and characters measured in this study, no subspecific categories of *D. milanjiana* should be recognized and may indicate a single or low number of introductions of this species in the Western Hemisphere.

Characteristics of the leaf epidermis and internal anatomy were studied using members of the annual complex and *D. milanjiana*. Obvious differences existed between the perennial and the annuals. Walls of long cells of *D. milanjiana* were straight to slightly wavy with rounded corners. Long cell walls of the annuals were undulating to sinuous with distinctly angled corners. Internal leaf anatomy showed that all specimens examined were C_4 subtype NADP-ME. Sclerenchyma tissue occurred over most of the vascular bundles in *D. milanjiana* but was associated only with first-order vascular bundles in the annual species.

A cytological comparison including embryo sac formation and pollen fertility was made between the *D. milanjiana* and the annual species. Numerous ovaries were examined in each species. Normal megasporogenesis of the polgyonum type was observed in each of the annual taxa. The mature embryo sac consisted of two synergids, one egg cell, two polar nuclei, and proliferating antipodals. Normal embryo sac formation was not observed in *D. milanjiana*. At some stage prior to anthesis the ovary withered and degenerated. This evidence suggests that this species is female sterile. Pollen fertility counts were made on 25 populations of the annual taxa and on four populations of *D. milanjiana*. The annuals were found to be highly male fertile, and no differences were observed among the taxa. Positive stains were observed on 94 to 99 percent of the pollen in each taxon. *Digitaria milanjiana* was found to be highly male sterile with approximately 1 percent of the pollen giving a positive stain. Apparently, in North America this species reproduces by runners and lateral branching from short, knotty rhizomes.

CONCLUSIONS

Digitaria milanjiana, a native of Africa, is an introduced species in North America and presently occurs in southern Texas, Florida, eastern Mexico, Central America, and the Caribbean. Based on taxonomically important characters within the annual complex, *D. milanjiana* is both phenetically distinct and statistically different from the most closely related annual species (*D. bicornis*). Instead of characters pertaining to the perennial nature of the species, it is suggested that absence of leaf pubescence, spicules on the mid-nerve of the lower lemma, lateral branch width, bract blade width, and the number of spikelets per unit area of lateral branch be used to distinguish *D. milanjiana* from the annual species.

Evidence presented in this study suggests that a measurable and definable difference exists between *D. milanjiana* and the annual members of the section *Digitaria*. The placement of *D. milanjiana* or any other member of the *Erianthae* into the section *Digitaria* is not warranted.

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